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Effects of Selenium and Zinc Enriched *Lactobacillus plantarum* SeZi on Blood Selenium and Zinc Concentrations, Antioxidant Capacities and Intestinal Flora in ICR Mouse

셀레늄 및 아연 고함유 *Lactobacillus plantarum* SeZi를 섭취한 ICR 마우스 혈액내의 셀레늄/아연 농도, 항산화 능력 및 장내균총 분석

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Abstract

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Selenium and Zinc are the trace minerals with multiple biological functions. However, the possible health benefits of the combined administration of dietary selenium and zinc have not been studied extensively. The aim of this study was to screen and prepare selenium- and zinc-enriched probiotic and investigate their effect on blood selenium and zinc concentrations, antioxidant capacities, and intestinal microflora in mice. After screening out and identifying 300 strains from human intestinal bacteria, it was found that *Lactobacillus plantarum* SeZi showed relative high tolerance to Na_2SeO_3 and $ZnSO_4 \cdot 7H_2O$ as well as the best bioaccumulation capabilities of selenium and zinc. To further assess the characteristics of this strain, the probiotic powder was prepared as follow. *L. plantarum* SeZi (1%) was inoculated and cultured in de Man Rogosa and Sharpe (MRS) broth with addition of 0.01 mM Na_2SeO_3 and 3.5 mM $ZnSO_4 \cdot 7H_2O$ at 37℃ for 24 h under anaerobic conditions. The

dry cell mass of the product was 2.5 g/l and *L. plantarum* SeZi biomass was 11.6 lg CFU/g. The inductively coupled plasma-atomic emission spectrometer (ICP-AES) was used to measure the concentrations of zinc and selenium. The results showed that 55.2 mg/kg selenium and 306.2 mg/kg zinc were detected in *L. plantarum* SeZi.

It was detected the genes coding these proteins including DedA, CysA, ZUR, ABC transporter, P-type ATPase family, cation diffusion facilitator family and MerR family from the whole genome sequencing of *L. plantarum* SeZi via Illumina MiSeq. The relatively high resistance and accumulation ability of *L. plantarum* SeZi with regard to selenium and zinc might result from the genes coding these proteins. Furthermore, safety evaluations of *L. plantarum* SeZi were performed via ammonia production test, hemolytic test and antibiotic susceptibility test. The results showed that *L. plantarum* SeZi didn't show ammonia production, hemolytic activity, nor transferable resistances.

Thirty-two male ICR mice were randomly assigned to four groups for a 30-day study. They were provided with a normal diet (C group) or a diet supplemented with Na_2SeO_3 and $ZnSO_4{\cdot}7H_2O$ (SZ group), *L. plantarum* SeZi (L group) or SeZn-enriched *L. plantarum* SeZi (LSZ group), respectively. Blood samples were collected immediately after scarification. Fecal samples were collected on day 0 and day 30. Compared with C group and L group, the concentrations of Se and Zn in the blood were significantly increased in the SeZn supplement group (SZ group and LSZ group). The increase of Se and Zn concentrations subsequently led to the increased activities of glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD) and the decreased blood malondialdehyde (MDA) level in the SZ group and LSZ group ($p<0.05$). As for the bacterial compositions investigated via 16S metagenome, the relative abundance of *Lactobacillus* in the feces were significantly increased, while *Bacteroides* were significantly decreased in the *L. plantarum* SeZi supplemented group (L group and LSZ group) ($p< 0.05$).

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Keywords: SeZn-enriched *Lactobacillus plantarum* SeZi, blood selenium and zinc concentration, antioxidant capacities, intestinal flora, mice

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1. Introduction

Selenium, as a vital trace element, exhibits antioxidant activities in a form of selenoenzymes such as glutathione peroxidase (GPX), selenoprotein P, thioredoxin reductase, methionine sulfoxide reductase and so on. Because of its antioxidant capacity in the body, selenium plays a role in the modulation of bone growth, regulation of antiviral capacity Rayman (2011) and prevention of disease, especially cancer (such as gastrointestinal cancer and liver cancer) and cardiovascular disease (such as hypertension and atherosclerosis) (Brenneisen, Steinbrenner, & Sies, 2005; Flores-Mateo, Navas-Acien, Pastor-Barriuso, & Guallar, 2006). Selenium deficiency can cause serious health issues such as Keshan disease, Kashin-Beck disease, poor growth, muscle pain and decreased immune response (Rayman, 2012). Selenium deficiency is mostly caused by receiving total parenteral nutrition and lacking selenium in soil. There are many areas of low soil concentration in the world including China, Central Africa and parts of Europe (Pophaly, Singh, Kumar, Tomar, & Singh, 2014). Selenium deficiency in soil is highly correlated with selenium deficiency in humans because crops grown on selenium-deficient soils have inherently low concentrations of selenium.

Zinc is also important for human health. Zinc is a key component of many metalloenzymes (i.e., superoxide dismutase (SOD), carbonic anhydrase, alcohol dehydrogenase) to maintain enzyme activity and various related metabolism in the body (Gropper & Smith, 2012). The function of zinc is mainly reflected in human growth, immunity, fertility and reproduction. Zinc deficiency in children can lead to growth retardation, abnormal bones, poor wound healing and delayed sexual maturity. Some of the symptoms and signs of adult deficiency include anorexia, diarrhea, lethargy, depression, hair loss and impaired immune function as well as wound healing (Hambidge, 2000). Zinc deficiency also occurs in many developed and developing countries primarily because almost half of the world's soil is zinc

deficient (Das & Green, 2016). In addition, some people, especially the elderly, low-income children, vegetarians and alcoholics, were found to be deficient in zinc (Gropper & Smith, 2012).

Selenium and zinc supplementation can correct the deficiency. In the past, it was common to incorporate selenium and zinc as inorganic forms (selenate, selenite, zinc oxide, zinc sulfate, zinc acetate, zinc chloride) or organic forms (selenomethionine, zinc gluconate). However, in recent decades, the use of microorganisms as trace element carriers has become a trend in this field. To be specific, trace elements are added to the medium during microbial fermentation. Then the microorganisms bind, take up and bioconvert the metal ions from the culture medium into an organic form lactic acid bacteria (LAB), which are important food-grade bacteria, have been extensively studied in this field. Mrvčić et al. have given an extensive review to emphasize the biosorption capacities of LAB towards metal ions including selenium and zinc (Mrv i , Stanzer, Šoli , & Stehlik-Tomas, 2012). Also, biotransformation of inorganic selenium into organic form in LAB was reported by Alzate, A., et al. (Alzate, Fernández-Fernández, Pérez-Conde, Gutiérrez, & Cámara, 2008).

Lactobacillus is one of the important genus of LAB. Stressful lifestyles and the consumption of pharmaceutical compounds, in particular antibiotics, could decrease the relative abundance of *Lactobacillus* in the gut. This decrease might cause the disruption of intestinal homeostasis and overall functionality (Fooks & Gibson, 2002; Rolfe, 2000). According to previous in vitro studies, *Lactobacillus plantarum*, isolated from fresh vegetables and fruit, showed a strong capacity to inhibit the growth of *Salmonella typhimurium* and *Escherichia coli*c*Listeria monocytogenes* (Trias, Bañeras, Badosa, & Montesinos, 2008). Moreover, the growth of *Helicobacter pylori* was found to be inhibited by Lactobacilli in the preclinical studies (Canducci et al., 2002). Thus, *Lactobacillus* have been widely applied in the

various food products to balance intestinal microbiota and protect our intestine from the related diseases.

Numerous previous studies have proved the transformation of zinc or selenium by LAB (Alzate et al., 2008; Mrv i et al., 2009) and the coeffect of Se and Zn on the immune system and antioxidant activities (Feren ík & Ebringer, 2003; Yan & Chang, 2012). However, only few papers reported the development of selenim and zinc enriched (SeZn-enriched) probiotic products. Therefore, the purpose of this study was to select and prepare SeZn-enriched *Lactobacillus* powder and investigate its effects on blood selenium and zinc concentrations, antioxidant capacities and the compositions of intestinal flora in mice.

2. Materials and Method

2.1 Screening and preparation of SeZn-enriched *Lactobacillus* strains

2.1.1 Bacterial strains and culture conditions

Human intestinal bacterial strains (300 strains) were isolated from Chinese and Korean human feces. All of the experimental bacteria were grown in de Man Rogosa and Sharpe (MRS) broth (Difco, USA) with L-cysteine · HCl (0.5 g/l) at 37℃ under an anaerobic atmosphere. All reagents were of analytical grade or higher purity. Na_2SeO_3 and $ZnSO_4 \cdot 7H_2O$ (Sigma, USA) were of analytical grade.

2.1.2 Screening and identification of *Lactobacillus*

When the concentration of selenium in the medium is high, strains convert inorganic selenium into element of selenium (red color) in the medium (Chaney & Marbach, 1962). In this experiment, 300 strains were inoculated into MRS agar supplemented with Na_2SeO_3 60 mM at 37℃ for 24 h under anaerobic condition. Strains resistant to high concentrations of Na_2SeO_3 were able to grow and appeared red in the agar plate. According to changing color of the agar plate, the strains with strong selenium resistance were selected.

Then the selected selenium-tolerant bacteria was used for zinc-resistant experiments. Some strains can grow with high concentration of zinc ions. However, some strains can not grow or grow slowly in broth with a high level of zinc ions. In this experiment, strains were inoculated into a broth of $ZnSO_4 \cdot 7H_2O$ 100 mM at 37℃ for 24 h under anaerobic condition. Strains with strong zinc tolerance were screened according to whether the growth of strains were inhibited. Next, the selected SeZn-tolerant bacteria were identified with 16sRNA sequence by Macrogen company in

Korea.

2.1.3 Enrichment of selenium and zinc in *Lactobacillus* during the cultivation

Considering that the strain may be used as food and feed additive application, the initial concentrations of Na_2SeO_3 and $ZnSO_4 \cdot 7H_2O$ were 0.01 mM and 3.5 mM, respectively (Yan & Chang, 2012). After 24 h of anaerobic culture in MRS broth, the bacterial cells were centrifuged (15600 × *g*, 5 mins) to gain pellet. Then it was washed three times with phosphate buffered saline (PBS), and frozen at -80℃ for lyophilization. The concentrations of zinc and selenium in the experimental bacteria were measured using an inductively coupled plasma-atomic emission spectrometer (ICP-AES, Optima-4300 DV, Perkin Elmer, Norwalk, CT, USA). Finally, the strain showing the best Se/Zn bioaccumulation capability was selected as experimental strain in the next experiments.

Se/Zn bioaccumulation rate were measured as follows:

Bioaccumulation rate of Se (%) = (Se content in dry cell mass / total Se content added in broth) × 100%

Bioaccumulation rate of Zn (%) = (Zn content in dry cell mass / total Zn content added in broth) × 100%

2.2 Gene analysis of Se/Zn uptake and resistance of experimental strain

The genomic DNA of the pure culture bacteria was extracted using MG™ Cell Genomic DNA Extraction SV Miniprep (MGmed, Korea), according to the manufacturer's instructions. Whole genome sequencing and analysis were completed using an Illumina MiSeq sequencer and a Nextera XT Library Preparation kit (Illumina, USA). Nextera XT sequencing indices were used for multiplexing, and the participants were free to choose any sample index combination. The run acceptance criteria were a sequencing output of 5.6 Gb (to achieve an average sequencing coverage of 100-fold for the 20 samples with genome sizes of 2.8 Mb) and a Q30 read quality score of 75% (Mellmann et al., 2017). The bioinformatics analysis was performed using Miseq raw data, and the comparative genomics analysis was completed with three Miseq raw data sets in ChunLab Co., Ltd. (Seoul, Korea).

2.3 Safety Evaluations of experimental strains

2.3.1 Ammonia production test

The experimental strains were incubated in BHI broth (Difco, USA) at 37℃ for 5 days. The ammonia production was determined by the method of Chany and Marbach (Chaney & Marbach, 1962). The culture supernatant of each strain was obtained by centrifugation at 10,000 × *g* for 30 mins at 4℃. The medium was then adjusted to pH 7 using 1 N NaOH. Two solutions were prepared as follows. Solution 1 is 200 ml of distilled water consisting of 2 g of phenol and 0.01 g of sodium nitroferricyanide dehydrate. Solution 2 is 200 ml of distilled water in which 1 g of sodium hydroxide and 0.08 g of sodium hypochlorite are dissolved. 100 µl of solutions 1 and 2 were distributed to 96 well plate with 10 µl of the supernatant of each strain. 96 Well plate was maintained at room temperature for 1 h and absorbance was measured at 625 nm. *Bifidobacterium bifidum* BGN4 was used as negative control (Kim et al., 2018). *Enterobacter cloacae* KCTC 2361 and *Enterococcus faecalis* KCTC 3511 were used as positive controls. Ammonia concentrations were calculated using standard curves. Three replicates of this experiment were performed on each strain.

2.3.2 Hemolytic test

The experimental strains were aerobically cultivated at 37℃ for 2 days in blood agar (BHI broth added with 1.5% agar and 5% horse blood). *Listeria ivanovii* subsp. *ivanovii* ATCC 19119 was cultured in the same conditions as a positive control for hemolysis (Kim et al., 2018). The presence of the hemolysis properties of microorganisms was analyzed by securing the plate to the light source and penetrating both sides of the plate. α-hemolysis, which produces a green-hued zone around the colony and γ-hemolysis, which does not cause hemolysis in the serum,

have been classified as non-hemolytic. Strains with white-hued zones around the colonies were classified as micro-organisms with hemolytic (β-hemolysis) properties.

2.3.3 Antibiotic susceptibility test

2.3.3.1 Antimicrobial agents

A total of 10 antibiotics purchased from Sigma, USA. They are clindamycin, gentamicin, vancomycin, kanamycin, streptomycin, tetracycline, ampicillin, chloramphenicol, neomycin and erythromycin. Each antibiotic powder was dissolved in sterilized distilled water, and then filter- sterilized to prepare various dilutions ranging from 1024 μg/ml to 0.0032 μg/ml. The prepared antibiotic diluents were sprayed 50 μl each into a 96-well plate.

2.3.3.2 Antibiotic susceptibility test

The minimum inhibitory concentration (MIC) values for all bacterial strains were conducted by ISO 10932: 2010 broth microdilution method (ISO 10932/IDF223). Colonies of experimental strain were suspended and diluted to correspond to an optical density value of 0.16 to 0.2 at 625 nm. Then experimental bacterial diluents were sprayed 0.3% in the double strength LSM-Cys broth, which was added 50 μl to each well in 96 well plate. Nextly, 50 μl of the above antibiotic diluents were completely mixed with the 50 μl 0.3% of the inoculated double strength LSM-Cys broth in 96well plate. Finally, the concentration of the inoculated bacteria was 1×10^5 cfu/ml. The resulting culture was incubated in an aerobic incubator at 37℃ for 48 h. This experiment was repeated 3 times.

2.3.3.3 Antibiotic resistance transferability test

Conjugal transfer of antibiotic resistance was assessed via the methods of Tannock (Tannock, 1987). Equal bacterial cell numbers (10^7) of the donor and recipient strains were mixed and centrifuged at 7000 × *g* for 10 min. After disposing

of the supernatant, the bacterial cell pellet was resuspended in the MRS broth medium and cultivated at 37°C for 18 h in an anaerobic chamber. The collected bacterial cells were filtered through a 0.45 μm microfilter membrane (Whatman Intl., UK) and the membrane was placed on the surface of MRS agar and incubated anaerobically at 37°C for 24 h. The bacterial cells were washed with 4 ml of 0.9% sterile saline, diluted to 10^{-3}, 10^{-4}, and 10^{-5}, respectively, and then plated on MRS agar containing erythromycin or neomycin. The plates were incubated aerobically or anaerobically at 37°C for 36 h. Three replicates of all experiments were conducted.

2.4 Effect of SeZn-enriched experimental strain on blood selenium and zinc concentrations, antioxidant capacities and intestinal microflora in ICR mouse

2.4.1 Preparation of SeZn-enriched experimental strain

The experimental strains were anaerobically grown at 37℃ for 24 h in MRS medium with the addition of 0.01 mM Na_2SeO_3 and 3.5 mM $ZnSO_4 \cdot 7H_2O$, the inoculation volume was 1%. For harvesting probiotic powder, the bacterial cells were centrifuged, washed, frozen and lyophilized.

2.4.2 Animals and diets

In our study, male ICR mice (7 weeks old) were purchased from Central Lab. Animal (Seoul, Korea). The animal breeding environment was adjusted to a dark cycle of 12 h light / 12 h dark at a temperature of 23 ± 1℃ and a humidity of 40-60%. After a one-week adaptation period, the mice were randomly divided into the following 4 groups (n = 8). C (a normal diet(ND) group); SZ: ND with Na_2SeO_3 and $ZnSO_4 \cdot 7H_2O$, selenium and zinc in the feed were 1.1 μg/g and 6.1 μg/g, respectively; L: ND with experimental strain, the number of viable bacteria per gram of feed was similar to the LSZ group; LSZ: ND with SeZn-enriched experimental strain. The mice were fed the experimental diets for 30 days.

2.4.3 Collection of samples

Blood samples were collected from the mouse heart into a 1.5 ml heparinized tube on day 30, and 0.9 ml of blood was centrifuged (840 × *g*, at 4℃, 10 mins) to obtain plasma. Whole blood and plasma samples were stored at -80°C for determination.

Fecal samples were collected under sterile conditions on days 0 and 30 and stored at -80°C.

2.4.4 Effect of different treatments on growth performance of mice analysis

The mice were individually weighed when grouped, and then weighed once on days 15 and 30 to record body weight.

2.4.5 Determination of Se and Zn concentrations in blood analysis

For determination of Se and Zn concentrations, the concentrations of Zn and Se in whole blood were measured using ICP-AES.

2.4.6 Antioxidant enzyme activities and malondialdehyde levels analysis

Antioxidant enzyme detection kits were purchased from the Nanjing Jiancheng Bioengineering Institute (Nanjing, China). GSH-Px activity in the whole blood, SOD activity in plasma, and malondialdehyde (MDA) levels in plasma were determined with the kits following the manufacturer's instructions.

2.4.7 Bacterial population of feces analysis

2.4.7.1 DNA extraction from sample collection

The microbial genomic DNA in stools was extracted using a QIAamp DNA Stool Mini Kit (QIAGEN, Hilden, Germany). The DNA samples were stored at -20℃ until analysis.

2.4.7.2 Gut microbiota analysis by 16s metagenomic sequencing

2.4.7.2.1 Library preparation

DNA concentration was measured using a Qubit® 3.0 Fluorometer (Thermo

Fisher ScientificTM, Waltham, MA, USA). The microbial genomic DNA (5 ng/ml) was amplified using the amplicon primers with overhang adapters attached targeting variable V3 and V4 region of the 16s rRNA gene. An Agencourt AMPure XP beads (Beckman Coulter, Brea, CA, USA) were used to purify 16S V3 and V4 amplicon away from free primers and primer dimer species. The 16S V3 and V4 amplicon was amplified with dual-index primers via PCR and purified with Agencourt AMPure XP beads. The index PCR amplicon possessed specific barcode sequences to distinguish among each other in the pooled library.

The concentrated library was measured using a Qubit® 3.0 Fluorometer (Thermo Fisher ScientificTM, Waltham, MA, USA) and diluted using Resuspension Buffer (RSB, Illumina, USA) to 4 nM. Five µl aliquot of diluted DNA from each samples were mixed for pooling libraries and 84 libraries were pooled for one MiSeq run.

2.4.7.2.2 Sequencing via an Illumina MiSeq platform

A pooled library was denatured with NaOH, diluted to 9 pM with hybridization buffer (HT1, Illumina, USA). A PhiX control library (Illumina, USA) was denatured with NaOH, diluted to 9 pM with HT1. The pooled library was mixed with the PhiX control (30%, v/v) and the combined library and PhiX control was loaded on a MiSeq® v2 (500 cycle) Reagent cartridge (Illumina, USA) for sequencing. All sequencing procedure were monitored through the Illumina BaseSpace® website.

2.4.7.2.3 Sequencing data processing

Sequence analysis, including demultiplexing and removal of indices, was performed using the bacterial metagenomics workflow in the MiSeq Reporter software (Illumina). The classification step uses a proprietary algorithm that provides each taxonomic level classification for paired-end reads.

2.5 Statistics Analysis

Data are expressed as mean ± standard deviation (SD). The results were compared using a nonparametric one way ANOVA test (Kruskal-Wallis H). Difference of $p<0.05$ were considered statistically significant. All data analysis was performed using IBM SPSS Statistics 22.0.

3. Result

3.1 Screening and preparation of SeZn-enriched *Lactobacillus* strains

Considering both selenium tolerance and zinc tolerance of *Lactobacillus*, four strains of *Lactobacillus* spp. (*L. plantarum, L. pentosus, L. fermentum, L. rhamnosus*) were screened among the about 300 experimental strains. Then the four prepared probotics powder was obtained after culturing in following culture conditions: the initial concentration of Na_2SeO_3 and $ZnSO_4 \cdot 7H_2O$ were 0.01 mM and 3.5 mM, respectively. Finally, *L. plantarum* SeZi yielded the greatest dry cell mass (2.5 g/l) as well as the best selenium bioaccumulation capability (19.5%) and zinc bioaccumulation capability (0.4%) among the four strains (Figure 1). Consequently, *L. plantarum* SeZi was selected and named as *Lactobacillus plantarum* SeZi for the next experiments.

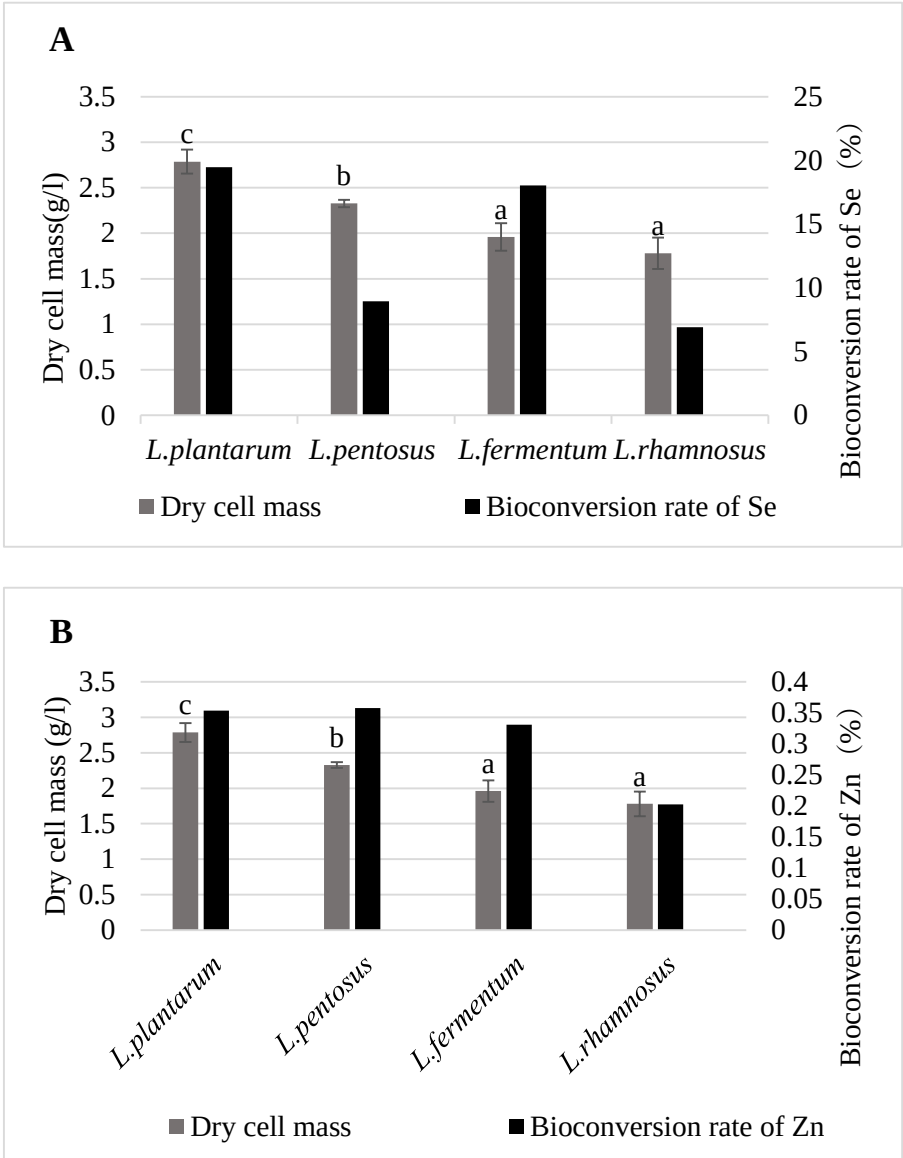


Figure 1. Dry cell mass (n=3) and the Se/Zn bioaccumulation rate of SeZn-enriched *Lactobacillus* spp. (A) Se and (B) Zn; Values are expressed as mean±SD; $p<0.05$

3.2 ene analysis of Se/Zn uptake and resistance of experimental strain

In order to identify possible genetic determinants involved in uptake and resistance of Na_2SeO_3 and $ZnSO_4 \cdot 7H_2O$ of *L. plantarum* SeZi, the whole genome sequencing (a total 2,223,664 bp circular chromosome (44.40% G+C) with 98.46% coverage) was read by means of Illumina MiSeq. As shown as table 1, the genes coding DedA protein and CysA protein that are related to Na_2SeO_3 uptaken were found in genome sequence of *L. plantarum* SeZi. It was also observed that the products of *L. plantarum* SeZi genome sequence contained zinc uptake regulation proteins ZUR and ABC transporter (Table 1) as well as zinc resistance proteins of P-type ATPase family, cation diffusion facilitator family and MerR family (Table 2).

Table 1. Gene products assumed to be related to the uptake of selenium in *L. plantarum* SeZi

Coding region[a]	Length (aa)	Product	Seed Function
264974-265621 (−)	648	Protein	Uptake of Na_2SeO_3, DedA protein
193218-193874 (−)	657	Protein	
19823-193874	894	Fe(3+)-transporting	Uptake of Na_2SeO_3,

a: Genes encoded on the minus strand were indicated with (−)

Table 2. Gene products assumed to be related to the uptake and resistance to zinc in *L. plantarum* SeZi

Coding region[a]	Length (aa)	Product	Seed Function	
237659-238102	444	Zinc specific metalloregulatory protein	Zinc uptake regulation protein	Uptake
98888-99691	804	Membrane protein ZnuB	Zinc import protein	
98203-98901	699	ATP-binding protein ZnuC	ABC transporter	
477531-479870	2340	ATPase exp7	Cation transport P-type ATPase family	Rsistan-ce
11986-12873 (-)	888	Probable zinc transporter MSC2	Cation diffusion facilitator family	
151-966	816	Multidrug-efflux transporter 1 regulator		
309784-310257	474	Uncharacterized HTH-type Transcriptional regulator		
342254-342718	465	Hypothetical protein		
54351-54800	450	Hypothetical protein		
22703-23146	444	Transcriptional activator mta	MerR family	
115660-116040	381	Transcriptional regulator		
27565-27957 (−)	393	Transcriptional regulator AdhR		
4022-4399	378	Transcriptional regulator ZntR		
227-619 (−)	393	Transcriptional regulator AdhR		

a: Genes encoded on the minus strand were indicated with (−)

3.3 Safety Evaluations of *L. plantarum* SeZi

3.3.1 Ammonia production test

In this study, *L. plantarum* SeZi did not produce ammonia. In contrast, *Enterobacter cloacae* KCTC 2361 and *Enterocus faecalis* KCTC 3511, used as positive control, produced 11.4 ± 0.9 μg/ml and 0.2 ± 0.6 μg/ml of ammonia, respectively. The *Bifidobacterium bifidum* BGN4, used as a negative control, produced no ammonia (Kim et al., 2018) (table 3).

Table 3. Ammonia levels of *L. plantarum* SeZi and other control bacteria (n = 3)

Strain	Ammonia (μg/ml)
Lactobacillus plantarum SeZi	negative
Bifidobacterium bifidum BGN4	negative
Enterobacter cloacae KCTC 2361	11.5 ± 0.9
Enterococcus faecalis KCTC 3511	0.2 ± 0.6

Values are expressed as mean±SD

3.3.2 Hemolytic test

In this study, the potential hemolytic activity of *L. plantarum* SeZi was evaluated using the platelet method. *Listeria ivanovii* subsp. *ivanovii* ATCC 19119 (positive control) showed a β-hemolytic clear area around the cell colonies. However, *L. plantarum* SeZi did not show hemolysis and there was no color change around the colonies (Figure 2).

(a) (b)

Figure 2. *L. plantarum* SeZi (a, back light) growth without blood cell lysis; Complete lysis of red blood cells was observed with clear regions around the *Listeria ivanovii* subsp. *ivanovii* ATCC 19119 (b, back light); positive control

3.3.3 Antibiotic susceptibility test

3.3.3.1 Antibiotic susceptibility

In order to distinguish antibiotic-resistant from antibiotic-susceptible microorganisms, the EFSA has established microbiological cut-off values for the antibiotic-resistance of microorganisms used as food and/or feed additives. These microbiological cut-off values were determined based on the distribution of the chosen antimicrobials' minimum inhibitory concentrations (MICs) in cell populations belonging to a single taxonomical unit (Additives & Feed, 2012).

L. plantarum SeZi was sensistive to cell wall synthesis inhibitors (ampicillin) and protein synthesis inhibitors (chloramphenicol, clindamycin and tetracycline), while showed a high level of resistance to glycopeptides (vancomycin). The MIC values of *L. plantarum* SeZi, except of erythromycin, were lower than the established EFSA cut-off values suggested by the EFSA's Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) (Additives & Feed, 2012). The susceptibility tendencies of *L. plantarum* SeZi was similar to other studies (Ammor, Flórez, & Mayo, 2007), with the exception of high MIC to erythromycin (MIC 4 μg/ml) in *L. plantarum* SeZi.

Table 4. Antimicrobial susceptibility (MIC values) of *L. plantarum* SeZi

Antibiotics	MIC values (μg/ml)	EFSA Microbiological cut-off values (μg/ml)
Clindamycin	0.25	2
Gentamicin	2	16
Vancomycin	>128	n.r.
Kanamycin	32	64
Streptomycin	16	n.r.
Tetracycline	16	32
Ampicillin	0.5	2
Chloramphenicol	4	8
Erythromycin	4	1
Neomycin	8	32

MIC = the minimal inhibitory concentrations; n.r. = not required

3.3.3.2 Antibiotic resistance transferability

Since *L. plantarum* SeZi showed high antibiotic resistance to erythromycin in these antibiotic susceptibility tests, erythromycin resistance transferability test was conducted using *Bifidobacterium bifidum* BGN4, a recipient strain that is highly susceptible to erythromycin. The conjugation results are shown in Table 5. *B. bifidum* BGN4, which is highly susceptible to erythromycin (MIC 0.125 μg/ml) while resistant to neomycin (MIC 1024 μg/ml) (Kim et al., 2018), grew well in MRS medium with 128 μg/ml neomycin; however, *B. bifidum* BGN4 did not grow in the MRS medium containing 0.5 μg/ml erythromycin. By contrast, *L. plantarum* SeZi showed resistance to 4 μg/ml erythromycin in this study. However, *L. plantarum* SeZi did not grow when cultured in the media containing 128 μg/ml neomycin. *B. bifidum* BGN4 did not grow in the media containing erythromycin after co-cultured with *L. plantarum* SeZi. Therefore, It was proved that *L. plantarum* SeZi′s resistance to erythromycin was not transferred to *B. bifidum* BGN4.

Table 5. Transferability of erythromycin resistance (*L. plantarum* SeZi) from donors to recipients (*B. bifidum* BGN4) (cfu/ml)

Antibiotics	SeZi		SeZi + BGN4		BGN4	
	Aerobic	Anaerobic	Aerobic	Anaerobic	Aerobic	Anaerobic
None[1]	1.48E+09	1.52E+09	3.70E+08	4.12E+08	0	9.83E+07
E0.5[2]	1.58E+09	1.40E+09	3.48E+08	3.63E+08	0	0
N128[3]	0	0	0	6.00E+07	0	7.33E+07

[1] No antbiotics were contained in the counting agar. [2] Erythromycin (0.5 μg/ml) was contained in the counting agar. [3] Neomycin (128 μg/ml) was contained in the counting agar. Values are expressed as mean±SD.

3.4 Effect of SeZn-enriched *L. plantarum* SeZi on blood selenium and zinc concentrations, antioxidant capacities and intestinal microflora in ICR mouse

3.4.1 Mouse diets

In the SeZn supplement groups (SZ group and LSZ group), selenium and zinc concentrations in mouse diets were 1.1 μg / g and 6.1 μg / g, respectively.

In the *L. plantarum* SeZi supplemented groups (L group and LSZ group), the numbers of bacteria per gram of feed were similar between two groups. In the bacterial powder, *L. plantarum* SeZi biomass and SeZn-enriched *L. plantarum* SeZi biomass were 11.6 lg CFU/g .

3.4.2 Effect of different treatments on growth performance of mice

As shown in Table 6, it can be concluded that there was no significant difference in the body weight between the four groups during the 30-day treatments.

Table 6. Effect of different treatments on weight performance of mice ($n = 8$)

Group(n=8)	Body weight (g)		
	0d	15d	30d
C	34.6 ± 0.9^{a}	39.3 ± 0.8^{b}	41.4 ± 2.1^{c}
SZ	34.5 ± 1.9^{a}	39.3 ± 2.4^{b}	40.1 ± 3.5^{c}
L	34.7 ± 2.1^{a}	39.2 ± 2.9^{b}	41.9 ± 2.7^{c}
LSZ	34.6 ± 1.8^{a}	38.5 ± 2.1^{b}	39.9 ± 2.8^{c}

Values are expressed as mean±SD; $p<0.05$

3.4.3 Determination of Se and Zn concentrations in blood

As shown in Table 7, zinc and selenium contents of mouse blood in the experimental group (SZ group and LSZ group) were significantly higher than that of the no administration group (C group and L group). In addition, there was no significant difference in Se concentration and Zn concentration between group SZ and group LSZ, group C and group L.

Table 7. Effect of different treatments on blood Se and Zn concentrations of mice (n=8)

Group(n=8)	Concentrations of whole blood(mg/l)	
	Se	Zn
C	0.3 ± 0.1^{a}	16.7 ± 9.3^{c}
SZ	0.4 ± 0.1^{b}	25.9 ± 5.9^{d}
L	0.3 ± 0.1^{a}	16.3 ± 7.9^{c}
LSZ	0.4 ± 0.1^{b}	30.8 ± 7.5^{d}

Values are expressed as mean±SD; $p<0.05$

3.4.4 Antioxidant enzyme activities and MDA levels

The result showed that the activities of the GSH-Px and SOD were significantly increased in the groups supplemented with selenium and zinc (SZ group and LSZ group). Especially, groups that ingested SeZn-enriched *L. plantarum* SeZi feeds showed a significant increase in the activity of GSH-Px. In addition, a significant decrease in the level of MDA was found in the SZ group and LSZ group (Table 8).

Table 8. Effect of different treatment on the blood antioxidant index of mice (n = 8)

Group(n=8)	GSH-Px (U/ml)	SOD (U/ml)	MDA (nM)
C	807.7 ± 91.6^{a}	133.3 ± 17.0^{x}	21.0 ± 4.0^{B}
SZ	1237.7 ± 202.8^{c}	146.5 ± 7.2^{y}	15.4 ± 1.8^{A}
L	976.5 ± 117.3^{b}	124.8 ± 9.1^{x}	20.2 ± 2.3^{B}
LSZ	1493.7 ± 118.2^{d}	150.4 ± 12.4^{y}	15.7 ± 3.1^{A}

Values are expressed as mean±SD; $p<0.05$

3.4.5 Bacterial population of feces

3.4.5.1 Bacterial OTU abundance in the phylum taxonomic level

Alterations in the phylum level of 0 day and 30 day fecal microbiota were compared inbetween control group with SZ group, L group and LSZ group. The ratio of Firmicutes/Bacteroidetes is used as a parameter to assess the composition of the intestinal microbiota (Ling et al., 2016). At the phylum level, the gut bacteria community was mostly dominated by Firmicutes and Bacteroidetes (Figure 3). The ratio between Firmicutes and Bacteroidetes of 0 day had no significant difference, whereas the ratio in *L. plantarum* SeZi added groups (L group and LSZ group) was increased significantly (Figure 4).

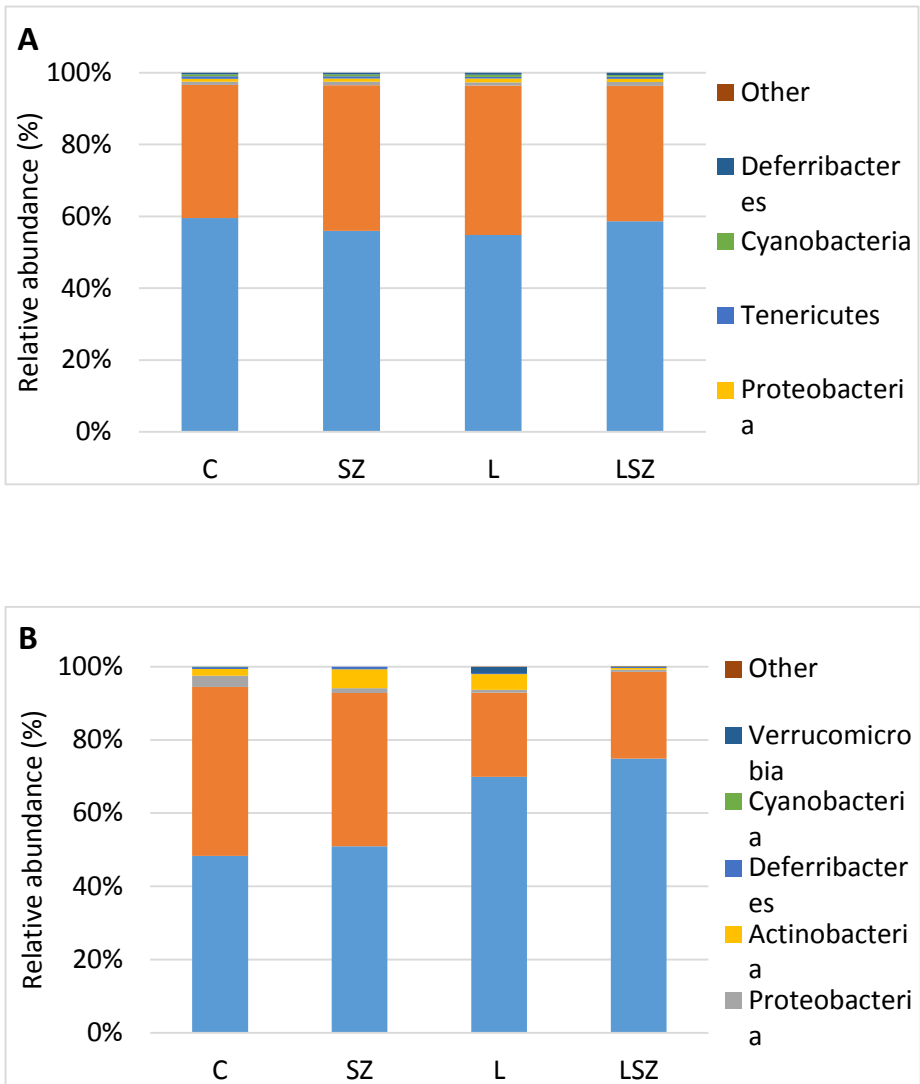


Figure 3. Overall microbiota composition of average relative abundance of each group at phyla level; (A) 0 day fecal samples and (B) 30 day fecal samples

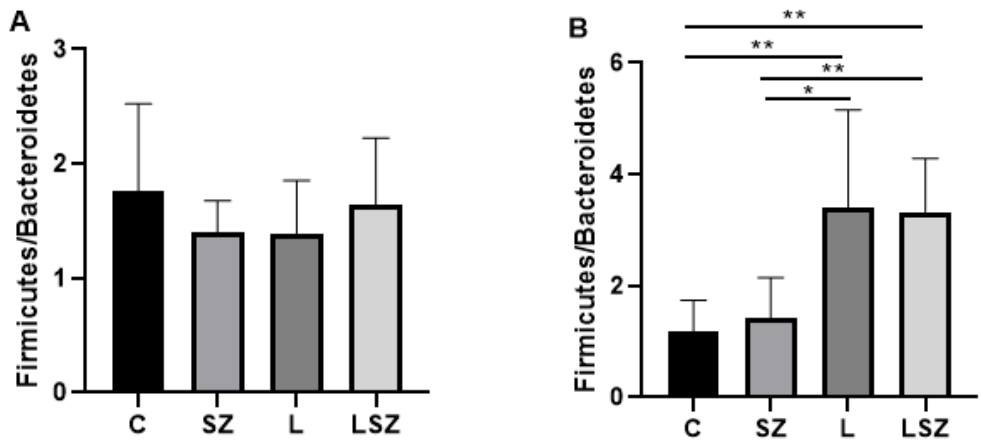


Figure 4. Ratio of Firmicutes:Bacteroidetes among groups; (A) 0 day fecal samples and (B) 30 day fecal samples.

* $p<0.05$, ** $p<0.01$, *** $p<0.001$

3.4.5.2 Different abundances of fecal microbiota composition between groups

To identify the key discriminative microbial taxa, the linear discriminant analysis effect size (LEfSe) was performed with an LDA value and Cladogram. As shown in Figure 5, LSZ group, supplemented with SeZn-enriched *L. plantarum* SeZi , was mainly characterized by *Lactobacillus* in the final day fecal microbiota. Remarkably, SZ group showed multiple bacterial clades. The addition of Na_2SeO_3 and $ZnSO4{\cdot}7H_2O$ in the diet resulted in a more enrichment of the *Lactococcus*, *Mucispirillum, Desulfovibrionales* as well as *Lachnospiraceae* and *Oscillospira* of *Clostridiales* in the SZ group. Moreover, in the control group, *Bacteroides* and *Enterobacteriaceae* showed the highest impact.

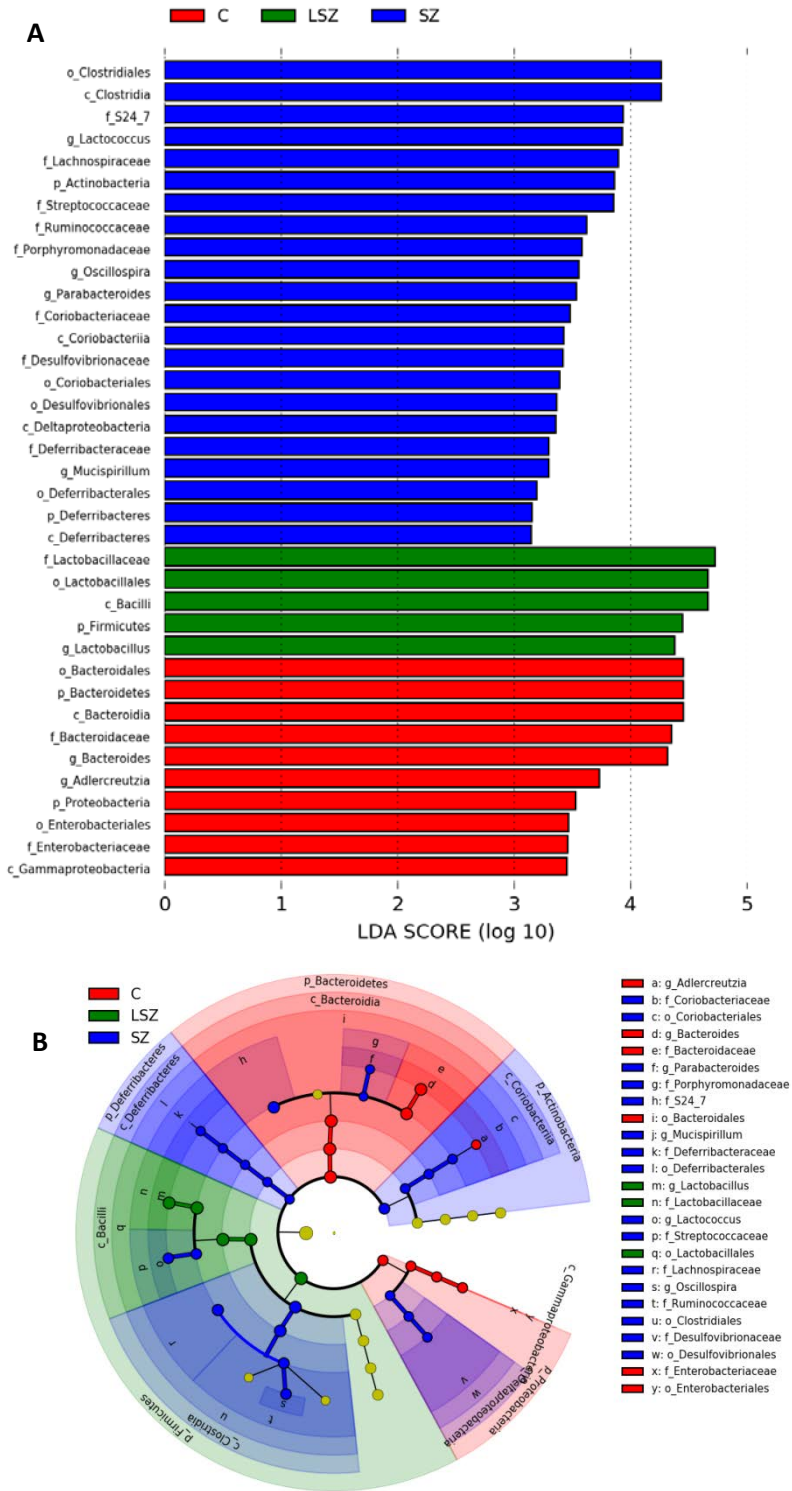
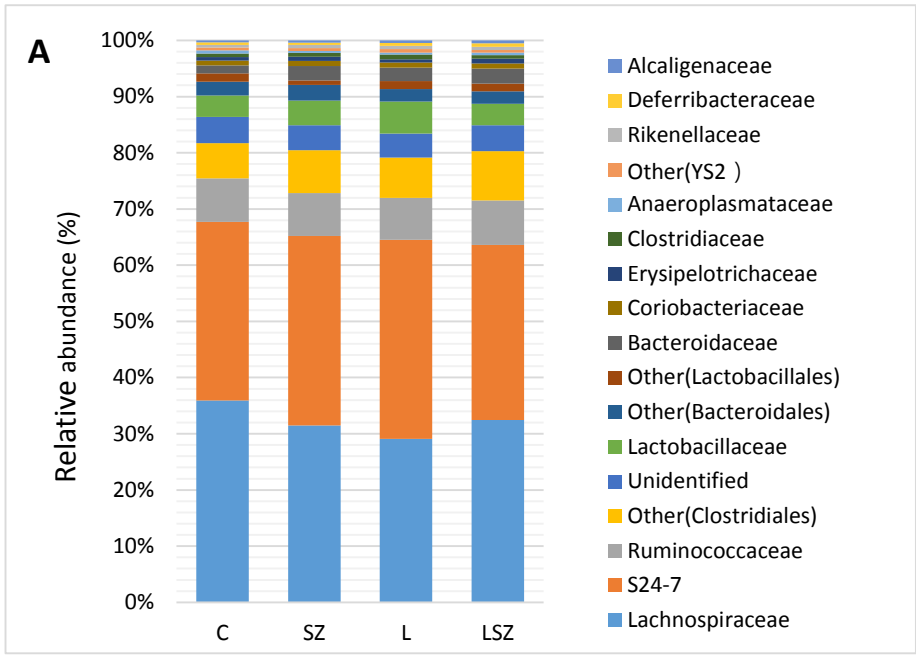


Figure 5. LEfSe analysis, showing taxa enriched in 30 day fecal microbiota among groups; (A) LDA scores and (B) Cladogram

3.4.5.3 Bacterial OTU abundances of taxa and changes in relative abundance of key bacteria

In this study, *Lactobacillus* were increased, whereas, *Bacteroides* were decreased in L group and LSZ group compared to C group and SZ group in 30 day. The relative abundances of *Bifidobacterium* in SZ group, L group and LSZ group was also increased compared to the control group but with no significant difference among the four groups. Therefore, *Lactobacillus* were one of the major contributors to the increase of Firmicutes in final day fecal microbiota in L group and LSZ group compared to control group (Figure 6, 7). In addition, *Lactococcus, Mucispirillum, Desulfovibrionales* as well as *Lachnospiraceae* and *Oscillospira* of *Clostridiales* were enriched in the SZ group based on the result of LEfSe analysis. It was found that in figure 8, compared to the other three groups, *Lactococcus* were increased significantly in SZ group after 30 days. However, other bacteria (*Mucispirillum, Desulfovibrionales* as well as *Lachnospiraceae* and *Oscillospira* of *Clostridiales*) did not showed significant changes in relative abundance compared to control group.



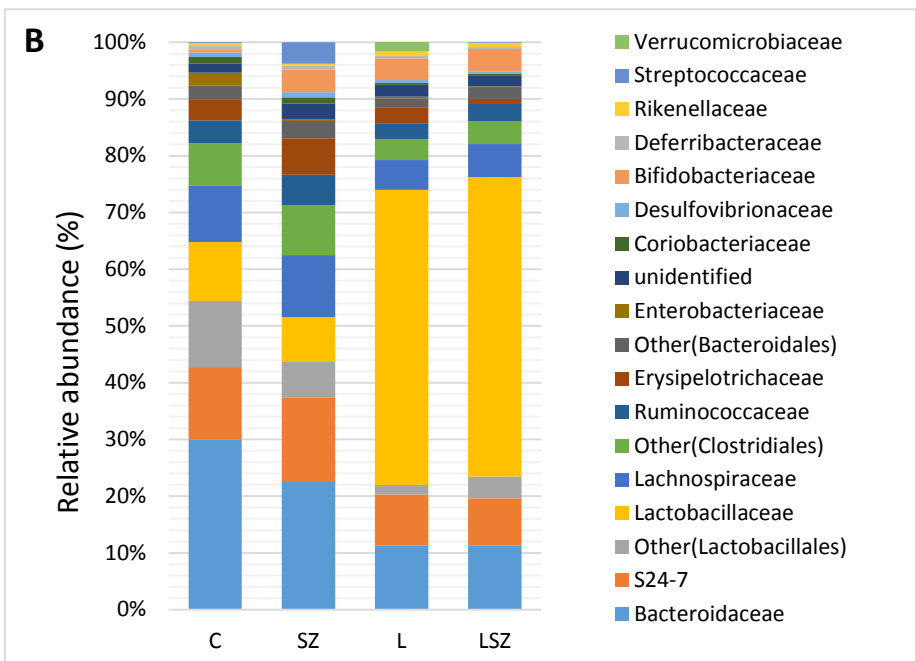
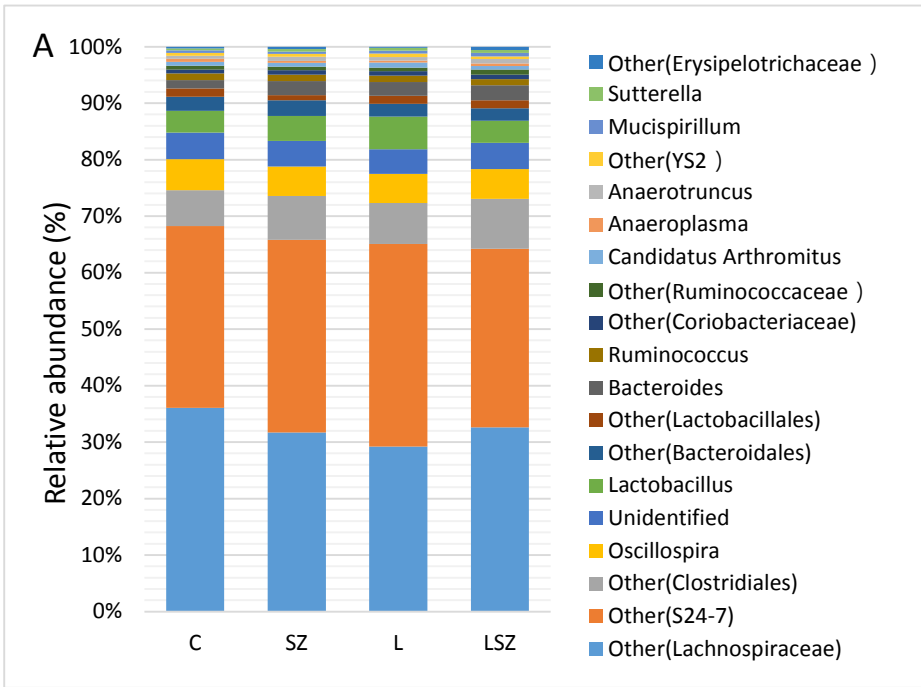


Figure 6. Overall microbiota composition of average relative abundance of each group at family level (>0.5%); (A) 0 day fecal samples and (B) 30 day fecal samples



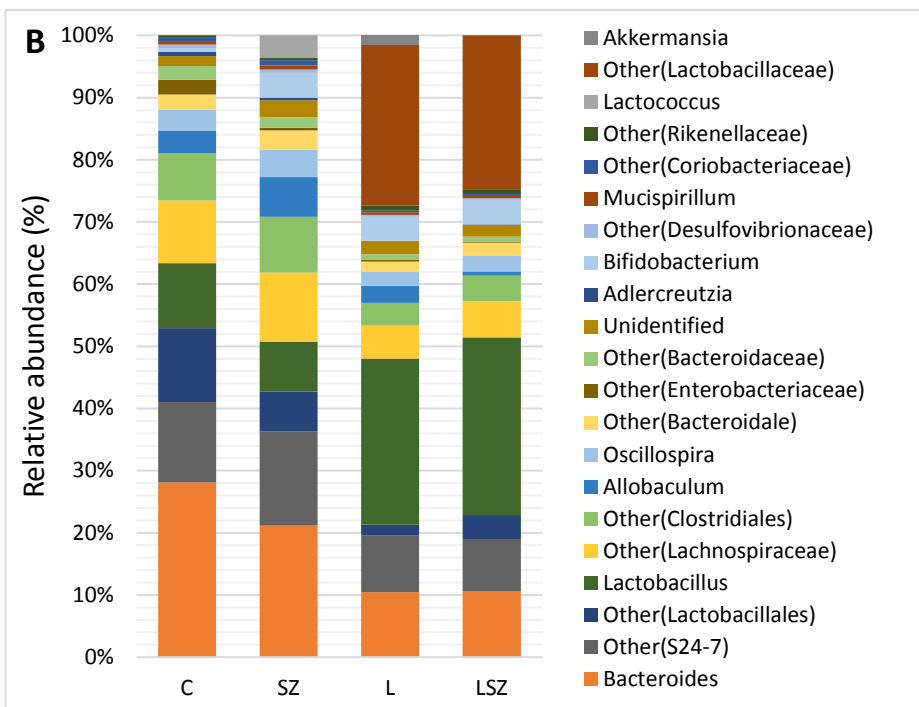
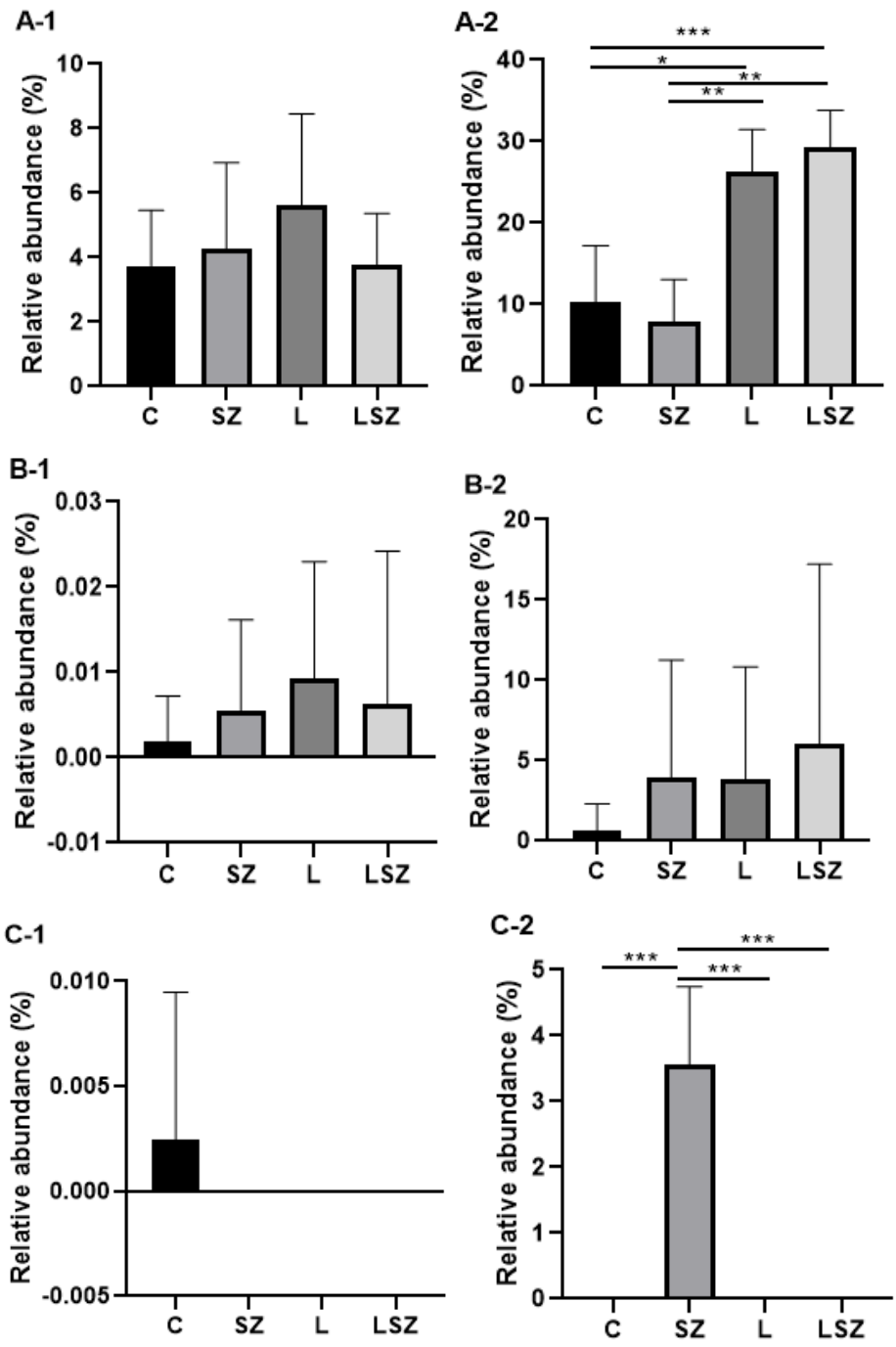


Figure 7. Overall microbiota composition of average relative abundance of each group at genus level (>0.5%); (A) 0 day fecal samples and (B) 30 day fecal samples


A-1
Relative abundance (%)
10
8
6
4
2
0
C
SZ
L
LSZ
A-2
Relative abundance (%)
40
30
20
10
0
***
*
**
**
C
SZ
L
LSZ
B-1
Relative abundance (%)
0.03
0.02
0.01
0.00
-0.01
C
SZ
L
LSZ
B-2
Relative abundance (%)
20
15
10
5
0
C
SZ
L
LSZ
C-1
Relative abundance (%)
0.010
0.005
0.000
-0.005
C
SZ
L
LSZ
C-2
Relative abundance (%)
5
4
3
2
1
0
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C
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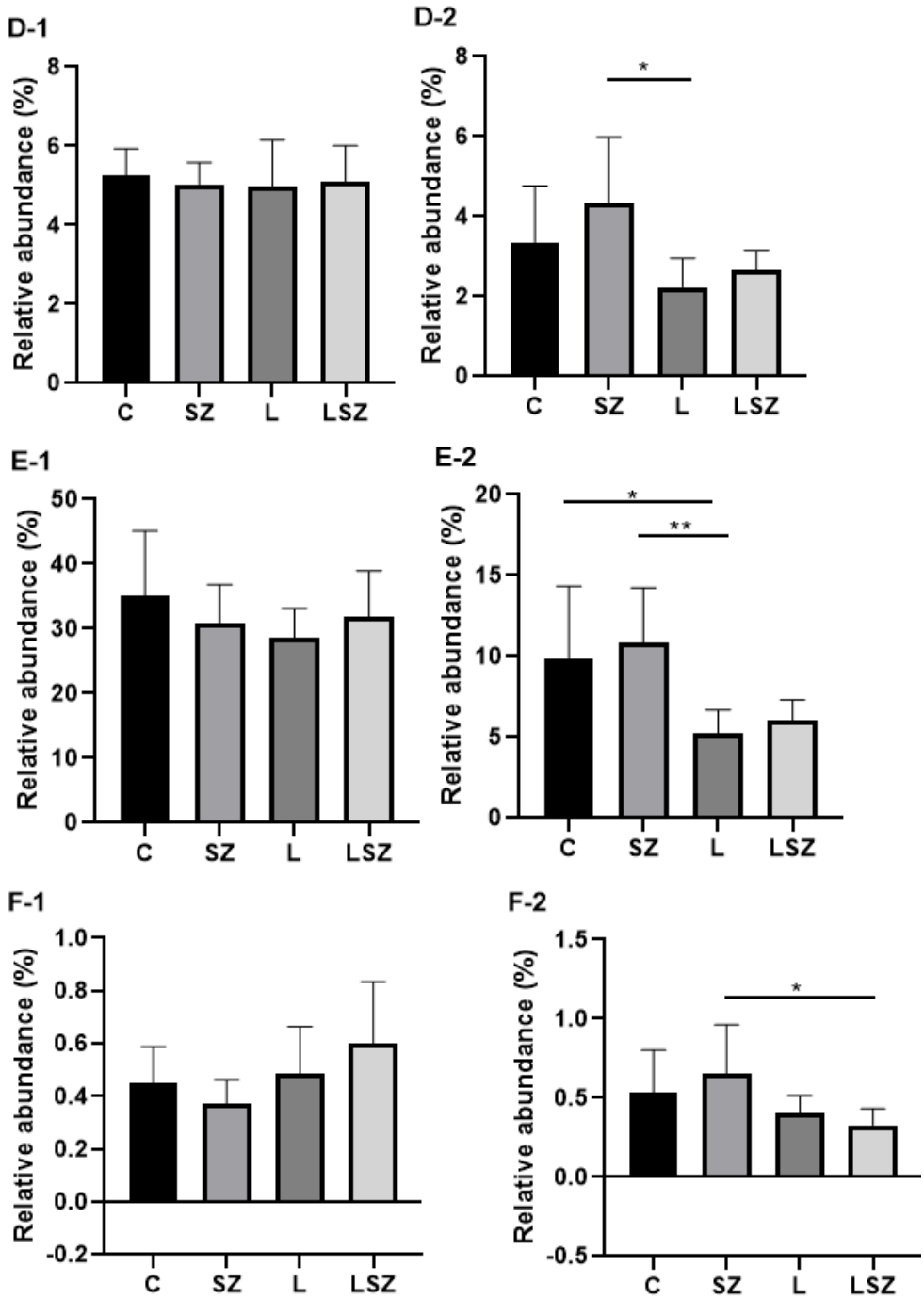
D-1
Relative abundance (%)
0
2
4
6
8
C
SZ
L
LSZ
D-2
Relative abundance (%)
*
0
2
4
6
8
C
SZ
L
LSZ
E-1
Relative abundance (%)
0
10
20
30
40
50
C
SZ
L
LSZ
E-2
Relative abundance (%)
*
**
0
5
10
15
20
C
SZ
L
LSZ
F-1
Relative abundance (%)
-0.2
0.0
0.2
0.4
0.6
0.8
1.0
C
SZ
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F-2
Relative abundance (%)
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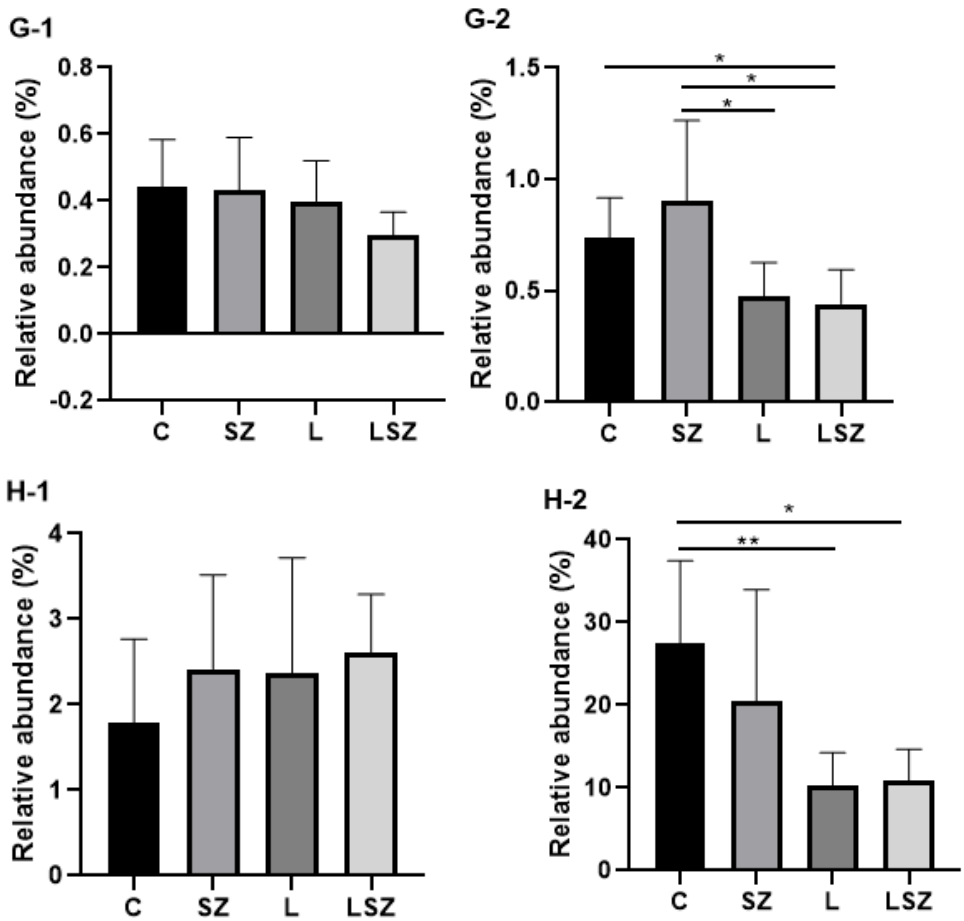

Figure 8. Composition of relative abundance of fecal bacteria among groups

(A) *Lactobacillus* (B) *Bifidobacterium* (C) *Lactococcus* (D) *Oscillospira* (E) *Lachnospiraceae* (F) *Mucispirillum* (G) *Desulfovibrionaceae* (H) *Bacteroides*; (1) 0 day fecal samplws (2) 30 day fecal samples

* $p<0.05$, ** $p<0.01$, *** $p<0.001$

4. Discussion

Micronutrient deficiencies, known as "hidden hunger", have affected more than 50% of the world's population (Mrv i et al., 2012). Different inorganic or organic forms of Zn or Se are supplemented to increase the daily intake of these minerals accordingly. Recently, research on the effect of probiotics enriched with essential element have attracted a lot of interest. The present study showed a noticeable resistance and accumulation ability of selenium and zinc in the selected *L. plantarum* SeZi. Likewise, Zinc uptaked by *L. plantarum* was previously studied by Jasna Mrvčić and coworkers (Mrv i et al., 2009). M. R. Calomme also reported that *L. plantarum* was able to concentrate selenium (Calomme, Van den Branden, & Vanden Berghe, 1995).

The accumulation capability and resistance to selenium and zinc of *L. plantarum* SeZi was probably caused by genetic determinants. In this study, the whole genome sequencing of *L. plantarum* SeZi indicated that this strain possessed the genes coding proteins of DedA, CysA, ZUR, ABC transporter, P-type ATPase family, cation diffusion facilitator family and MerR family.

DedA was related to selenite uptake, either as a direct transporter or as a helper in the selenite transport (Ledgham, Quest, Vallaeys, Mergeay, & Covès, 2005). Furhermore, selenate may enter the cells of *E. coli* and *Saccharomyces cerevisiae* through the sulfate permease CysA (Smith, Hawkesford, Prosser, & Clarkson, 1995; Turner, Weiner, & Taylor, 1998). If bacteria grow at low concentrations of selenium (<0.05 mM), most of the selenium uptaken and accumulated by the bacteria is possibly in organic form (Zhang et al., 2009). On the other hand, many Lactobacilli are able to resist high concentration of selenite by uptaking and reducing to elemental selenium (Se(0)) because Se(0) is chemically stable, insoluble and essentially non-toxic (Pophaly et al., 2014).

Maintaining zinc homeostasis in bacteria is an important way for bacteria to detoxify and store zinc. Zn^{2+} homeostasis in bacteria is achieved by uptake systems and export systems which are separately regulated by their own regulators. The high-affinity zinc uptake was provided by binding proteins from ABC transporters group, regulated mostly by Zur protein, while multi-drug efflux transporters, P-type ATPases and cation-diffusion facilitators are the three most frequently types of Zn^{2+} export systems that protect cells from high toxic concentrations. The exporters were regulated in bacteria by MerR-like repressors and ArsR-like repressors (Hantke, 2001).

Many *L. plantarum* are widely used as a probiotic in fermented products and medicine around the world because of their various benefits. However, they need to be used discriminately with scientific evidence or safety verification (FAO/WHO, 2002). In this study, the safety of *L. plantarum* SeZi was evaluated via ammonia production test, hemolytic test and antibiotic susceptibility test.

Gut bacteria can degrade various nitrogen sources found in intestinal feces to produce various toxic substances (i.e., phenol, ammonia and indole) (Igai et al., 2016). Thus, bacterial ammonia production is highly correlated with human intestinal health. In the study, the *L. plantarum* SeZi strain did not produce ammonia. This is consistent with the study results of Vince, A.J. et al. (Vince & Burridge, 1980).

Microbial hemolysis properties could result in anemia and edema in the host. In this study, the potential hemolytic activity of *L. plantarum* SeZi was assessed using blood agar plate inoculation. It was found that hemolysis and color change were not observed on the periphery of the colony of *L. plantarum* SeZi .

The problem of pathogens' resistance to antibiotics has been developed seriously. LAB could possess the potential risk of transferring the resistance gene to pathogens (Borriello et al., 2003). Thus, the determination and assessment of the

resistance of LAB are important for safety evaluation. The antibiotic susceptibility test herein found that *L. plantarum* SeZi was sensitive to clindamycin, gentamicin, vancomycin, kanamycin, streptomycin, tetracycline, ampicillin, chloramphenicol, neomycin except erythromycin. However, the erythromycin resistance of *L. plantarum* SeZi was not transferred to the recipient, *B. bifidum* BGN4, in this study. Therefore, this proves *L. plantarum* SeZi′s resistance to erythromycin can not transferred to the recipient strains.

Generally, the selenium and zinc concentration of the whole blood are usually maintained at certain range, but they can be affected by dietary supplementation and the related metabolism in the body. Based on the research of Yan H and Chang H, in which the mice in experimental group received about 300 μg of selenium and 1.5 mg of zinc per kg body weight per day (Yan & Chang, 2012), In the in-vivo study, the effect of supplementation of selenium 1.1 μg/g and zinc 6.1 μg/g in the mouse diet were determined. On 30 day, the blood concentrations of selenium and zinc in SeZn added groups (SZ group and LSZ group) were increased significantly compared to control group. It was reported that both selenium yeast and Na_2SeO_3 increased plasma selenium in healthy Finnish men after they intaked selenium resulting to the increases from 40 μg/d to 100 μg/d for 11 weeks (Alfthan, Aro, Arvilommi, & Huttunen, 1991). Another study also proved that the blood selenium and zinc concentrations in canine were increased after feeding a diet supplemented with 2.0 g of Se/Zn-enriched *Lactobacillus* for 35 days (Ren, Zhao, Wang, & Huang, 2011).

Selenoproteins (e.i., GPx, selenoprotein P, thioredoxin reductase, methionine sulfoxide reductase) and zinc dependent proteins (e.i., SOD) domonstrate antioxidant capability mainly by removing superoxide radicals and peroxides produced in the body. Superoxide radicals and peroxides can destroy the unsaturated double bonds of phospholipids in the membrane structure, resulting in increased lipid peroxidation of the membrane. MDA is the most important product of membrane

lipid peroxidation. Thus, GSH-Px, SOD and MDA levels are important indicators for assessing antioxidant capacities (Child, Wilkinson, Fallowfield, & Donnelly, 1998). As shown in this study, selenium and zinc supplementation in the mouse diet increased not only concentrations of selenium and zinc in the blood, but also antioxidant capacities. The antioxidant functions of selenium and zinc which were associated with the supplementation of selenium and zinc enhanced activity of host metalloenzymes (Pophaly et al., 2014). The increased activity of GSH-Px and SOD as well as the decreased MDA level were also proved after feeding Zn-enriched oyster mushroom or SeZn-enriched *Lactobacillus* (Ren et al., 2011; Yan & Chang, 2012).

Finally, we used the Next Generation Sequencing (NGS) method to determine whether SeZn-enriched *L. plantarum* SeZi contributed to the beneficial alterations in gut microbiota when it was supplemented in the mouse diet. In this study Firmicutes and Bacteroidetes were predominant. Firmicutes were increased whereas Bacteroidetes were decreased in *L. plantarum* SeZi added groups (L group and LSZ group). *L. plantarum* ZDY 2013 administrated in mouse diet for three weeks showed the positive effect in changing the ratio between Firmicutes and Bacteroidetes in intestinal microbiota (Pan et al., 2016). Furthermore, in the study of human dietary intervention, complex probiotic preparation was proved to increase the ratio of Firmicutes/Bacteroidetes in fecal microbiota (Yoon et al., 2015).

The key bacteria at the family and genus levels of intestinal flora were analyzed according to the LEfSe analysis and bacterial OTU abundances of taxa. The relative abundance of *Lactobacillus* was significantly increased in L group and LSZ group because of the addition of SeZn-enriched *L. plantarum* SeZi in diet. Similiar result was found by Ren, Z., et al. (Ren et al., 2011). However, *Bacteroides* were significantly decreased in the *L. plantarum* SeZi supplemented group in 30 day. Bacteriocins produced by *Lactobacillus* are active against a wide range of food-

borne pathogens (Kailasapathy & Chin, 2000). Futhermore, lactic acid from *Lactobacillus* metabolism are able to reduce intestinal pH and Eh (redox potential) values, which is beneficial to inhibit the growth of pathogenic microorganisms in the gastrointestinal tract (Ren et al., 2011). *Bacteroides* are opportunistic pathogen, and it often co-exist with other harmful bacteria which can lead to endogenous infections in the event of immune dysfunction. Opportunistic bacteria would harm people when the body turns into a weakened state (Wang et al., 2017). Thus, *Bacteroides* in mouse feces might have been significantly dereased in L group and LSZ group by the produced bacteriocins and lactic acid. Furthermore, it was newly found that the gut microbiota of the whole 8 ICR mice in SZ group showed the highest relative abundance (3.6%) of *Lactococcus* among the four groups, which was mainly correlated with the addition of Na_2SeO_3 and $ZnSO_4 \cdot 7H_2O$ in the diet.

In summary, *L. plantarum* SeZi showed a relatively high SeZn resistance and accumulation ability of selenium and zinc, which could be genetically explained the genes coding proteins of DedA, CysA, ZUR, ABC transporter, P-type ATPase family, cation diffusion facilitator family and MerR family could showed roles in the uptake and resistance of selenium and zinc. In safety evaluations, *L. plantarum* SeZi showed little risk of harmful effects in ammonia production test, hemolysis test and antibacterial susceptibility test. *In vivo*, *L. plantarum* SeZi increased blood Se/Zn concentrations and antioxidant capacities and showed an beneficial alterations of microbiota by promoting *Lactobacillus* and suppressing *Bacteroides*. Consequently, the benefits of this product are amalgamation of individual role of Zn species, Se species and *Lactobacillus*.

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국 문 초 록

셀레늄과 아연은 항산화능을 가진 필수 미량원소이다. 본 연구의 목적은 셀레늄과 아연이 풍부한 유산균을 선별하고 이를 마우스에 섭취시켰을 때 혈액 내 셀레늄과 아연의 농도, 항산화 능력 및 장내균총 분포에 미치는 영향을 연구하는 것이다.

본 연구에서 사용된 300 개의 균주 중에서 셀레늄과 아연을 가장 많이 축적한 균주는 *Lactobacillus plantarum* 이었다. 따라서 이 균주를 *Lacto- bacillus plantarum* SeZi로 명명하였다. 먼저, 셀레늄과 아연에 대한 저항성과 축적능력에 관련된 유전자 정보를 식별하기 위해 *L. plantarum* SeZi를 대상으로 whole genome sequencing을 수행하였다. *L. plantarum* SeZi 의 genome 에서 무기 셀레늄인 selenite 의 섭취와 관련된 단백질인 DedA , CysA , 아연 섭취 조절 단백질인 ZUR, ABC transporter 와 내성 관련 단백질인 P-type ATPase family, cation diffusion facilitator family, MerR family의 유전자들이 발견되었다. 해당 균이 지닌 셀레늄과 아연에 대한 저항성과 축적능력은 아마도 이러한 유전자들이 존재하기 때문인 것으로 사료된다. 해당 균의 안전성을 평가해보았을 때, *L. plantarum* SeZi 는 암모니아를 생산하지 않았고 용혈성을 나타내지 않았으며 항생제 내성을 전달할 수 없었다. 다음으로 *L. plantarum* SeZi를 섭취하였을 때 체내에 미치는 영향을 확인하고자 동물실험을 수행하였다. 수컷 ICR 마우스를 사용하였으며 32 마리를 무작위로 4 개 군으로 나누었다. C군은 일반 식이를 먹인 군이며, SZ군은 Na_2SeO_3와 $ZnSO_4 \cdot 7H_2O$ 를 사료에 첨가한 군, L 군은 *L. plantarum* SeZi 를 사료에 첨가한 군, LSZ 군은 셀레늄과 아연을 함유한 *L. plantarum* SeZi 를 사료에 첨가한 군이다. 이 중 SZ 군과 LSZ 군의 사료에 첨가된 셀레늄과 아연의 함량이 같았고, L 군과 LSZ 군의 사료에

첨가된 유산균의 양이 동일하였다. 30 일 동안 4 개 군 사이의 마우스 체중에 유의적인 차이가 관찰되지 않았다. 사료에 아연과 셀레늄을 첨가한 실험군 (SZ 군와 LSZ 군) 마우스의 혈중 아연과 셀레늄 함량은 아연과 셀레늄을 첨가하지 않은 군보다 높았다. 항산화 실험 결과에서는 SZ 군과 LSZ군의 glutathione peroxidase 와 superoxide dismutase 의 활성이 증가하였고 malondialdehyde 수준이 감소하였다. 다음으로 마우스의 변을 대상으로 장내균총 분석을 수행하였다. Phylum 수준의 경우, 실험 시작 0 일째에는 실험군 간의 Firmicutes와 Bacteroidetes의 비율이 큰 차이가 없었던 반면, 30 일이 지난 후 *L. plantarum* SeZi를 섭취한 군 (L 군과 LSZ 군)에서의 Firmicutes 와 Bacteroidetes 의 비율은 다른 군들에 비해 유의적으로 증가하였다. Genus 수준의 경우, 30 일 동안 다른 군들에 비해 L 군과 LSZ 군의 *Lactobacillus* abundance가 증가하였고 *Bacteroides* abundance가 감소하였다.

본 연구에서 *L. plantarum* SeZi가 셀레늄과 아연에 대한 높은 내성 및 축적 능력을 지니고 있는 것으로 확인되었다. 해당 균의 안전성 평가에서 *L. plantarum* SeZi 는 암모니아 생산 시험, 용혈 시험 및 항생제 민감성 시험에서 유해성이 관찰되지 않았다. 동물실험에서 *L. plantarum* SeZi 는 혈중 셀레늄과 아연의 농도를 증가시키고 항산화 능력을 향상시켰으며, 장내균총에서 *Lactobacillus* 의 증가를 촉진하고 *Bacteroides*를 감소시키는 유익한 변화를 가져왔다.

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주요어 : 셀레늄 및 아연 고함유 *Lactobacillus plantarum* SeZi, 혈액 셀레늄과 아연 농도, 항산화 능력, 장내균총, ICR 마우스

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